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INFECTION AND REPRODUCTIVE OUTCOME

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The relation between infection and reproductive outcome involves several problems regarding the decrement of fertility due to the inflammatory reaction in the female pelvis. Moreover another important issue concern the risk of transmission to the partner and to the embryo. For those reasons all the couple with infertility or recurrent miscarriage should be screened for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), rubella, syphilis, and Chlamydia IgG and IgA serology; TPHA-VDRL. It could be suggested also to test for Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum, and Candida albicans, a vaginal and endocervical microbiological samples from female and a microbiological sperm samples from the male. In recent years, the rate of survival and expectation of life of people with chronic infections disease has dramatically improved and this has leaded to an increased demand for ART (Assisted Reproduction Tecniques). We well know the main positive factors that contribute to successful *in vitro* fertilization (IVF) cycles are to one side maternal age, the oocytes the number and quality of transferred embryos to embryoloby lab. It has become critically important that appropriate guidelines be adopted to handle these cases and to manage infection avoiding risk of contamination and infection. The purpose of this paper is to describe issues relating to infection during IVF and to propose guidelines to control infection and to increase laboratory safety.

HEPATITIS B VIRUS

From the epidemiological point of view, the prevalence of HBV infection in the general population is 2% in western Europe and the USA, 2-7% in eastern Europe and South America, and 7% in Africa and Asia. In Italy, considering the population between 40 and 60 years, 40% have positive markers. In all pregnant women the prevalence is 1.6%. The prevalence in reproductive-age women is 1-2%, but there is great risk of diffusion from the infected male: more than 50% of partners are infected or seropositive. Chronic infection does not seem to decrement fertility or to have adverse effects on pregnancy and the pregnancy does not influence the course of hepatitis B infection.HBV is mainly transmitted through mucosal exposure to the blood or other fluids (saliva, bile, nasal secretion, human milk, sperm, vaginal mucus) of infected people. Therefore, the transmission occurs mostly through parenteral means, by sexual intercourse, but also via saliva. This virus has a parenteral spread and a high infective potential even after exsiccation out of the body.

- If, in a couple, one or both partners are positive for HBsAg, it is necessary to screen for all the serologic markers for hepatitis B (HBsAg, HBeAg, HBV DNA virus). The probability of transmission from a women to her child is ~15% if she is HBsAg positive, but ~90% if she is HBsAg positive and HBeAg positive or HBV DNA positive.
- If the woman is positive for HBsAg but all other criteria are negative and the male partner is negative, it is recommended that the partner be vaccinated and informed of the risks for vertical transmission (perinatal prophylaxis is required).
- When the man is positive for HBsAg but all other criteria are negative, it is important for him to understand that he is able to transmit the infection sexually and that is relevant the woman is vaccinated and antibody titers (anti-HBs >10 mU/mL) requested. Furthermore, there are risks of infection for the operators during the sperm treatment process, so strict precautionary measures are necessary.

HEPATITIS C VIRUS

Acute HCV infection is often asymptomatic and it is rarely associated with icteric hepatitis. Transmission can occur by symptomatic subjects with acute or chronic hepatitis, but mostly it occurs via asymptomatic infected carriers with no clinical signs of infection or alterations of

hematological markers. Several studies evaluated HCV prevalence in the general population in Italy and they show many variations by zone and age (in particular, the range was 2.4% of Lazio to 16.3% of Campania). Therefore, it is only possible to approximate the prevalence in Italy. The incidence is 1 in 100,000. HCV has low sexual transmission rates. In fact, the prevalence of anti-HCV antibodies in the partners of HCV subjects is slightly above the average of the general population. Vertical transmission occurs in 5% of cases of high viremia. Intravenous drug use and association with HIV increase the risk of fetal and neonatal infection.

It is important to suggest a preconceptive control in the couple because it is possible to eradicate the woman's infection before pregnancy (by pharmacologic treatment with interferon), the effectiveness of elective cesarean to reduce transmission has yet to be proved, and it is possible to transmit the virus via the sperm of an infected man.

- If the woman is anti-HCV positive, we should test the virus-positive results with a RIBA or qualitative PCR; if plasmatic HCV-RNA is negative, there is no viremia and therefore no vertical transmission; if the hepatitis is chronic, generally there are no contraindications to hormonal administration or pregnancy. (2)
- If the man is anti-HCV positive the dosage of viremia is less important as few cases with viremia have HCV-RNA in the sperm or inside the spermatozoa, but there are differences between the studies. French studies suggest that the presence of HCV RNA in the sperm could be an indication for serologic testing of the woman with an infected partner after insemination or embryo transfer. However, current data do not indicate that ARTs are necessary to prevent infection of a woman by her HCV-infected partner. (3)
- If the woman is HCV positive, we must also evaluate RNA: if the RNA is negative, no problem exists; if the RNA is positive, there is a 5% risk of vertical transmission.

  Usually the disease in the newborn is chronic and benign, and there are few important cases of hepatitis. No case of activations of maternal hepatitis has been reported. For HCV-positive patients a consultation with an infectious diseases specialist is recommended and the interferon + ribavirin therapy is considered suitable and effective before ART (60-80% in some types of virus). This therapy is contraindicated during pregnancy and ART must delay 7 months after therapy. (4)
- When the man is HCV positive, there are few data regarding fetal infection from spermatozoon, and the rate of sexual transmission is very low if the hepatitis is chronic (0.41%), it is important to detect the viremia by the PCR technique. It has been showed that sperm preparation following the Semprini protocol is effective in removing both HCV and HIV viruses and after that it can be suggested the most suitable ART programme.

HUMAN IMMUNODEFICIENCY VIRUS

HIV affects 40 million people throughout the world. In Italy, there are about 100,000 people infected, 33% of them are women. Of infected people, 95% are between 20 and 49 years of age (maximal incidence: 30.8% between 30-34 years).

- If the woman is positive and the partner is HIV negative, the risk of HIV transmission to the man can be eliminated using homologous insemination with his sperm. In asymptomatic women whose infection is well controlled, pregnancy does not aggravate HIV disease. (14,15) Amniocentesis is strongly contraindicated, because of the high risk of vertical transmission
- Untreated HIV-positive women have a risk of vertical transmission greater than 20% in relation to viremia, but the treatment with zidovudine during the delivery and further administration of zidovudine to the newborn child for 6 weeks reduce the risk to 5-8%. Delivery by cesarean section and no breastfeeding reduce further the risk to 2%. Optimization of antiretroviral therapy of the mother with viremia HIV negative (< 40 copies/mL) increases that chance of having an uninfected baby.
- If the man is positive, the use of condoms during sexual intercourse, except during the ovulatory period, can reduce the risk of infecting the woman, but it does not eliminate it (seroconversionindex is 4.3%), and therefore this method is not recommended. In 1998, Semprini and colleaguesusing a density gradient and other techniques for washing sperm, obtained sperm with no measurable levels of HIV by PCR and used these washed sperm in ART techniques. To

reduce the possibility of infection, it is always important to offer highly active antiretroviral therapy (HAART) which decreases the viremia of the infected partner. It is also important to remember that blood viral count does not always correlate with sperm viral count because the virus segregates into different compartments and finds some glands a better habitat than others. (16)

- When both man and woman are positive and the viremia is reduced to "undetectable" in both partners (less then 40 copies/ mL), the couple can have an HIV-negative child (risk 2%). More aggressive anti-HIV therapies have remarkably extended life expectancy and ethics problems are similar to those of couples that are carriers of recessive autosomal genetic damage (25% risk) or with falciform anemia or cystic fibrosis who adopt a child or use donor gametes.

CHLAMYDIA TRACHOMATIS

C. trachomatis genital infection is a common sexually transmitted bacterial infection. Because a high percentage of infected subjects are asymptomatic (70% of women and 75% of men), it is estimated that only 10% of the infections are diagnosed. Yet, untreated infection can lead to severe complications, such as chronic pelvic pain, pelvic inflammatory disease, conditions related to ectopic pregnancies, subfertility or even infertility.

Moreover, infection is associated with an increased risk of HIV transmission. The prevalence of infection depends on the characteristics of the population studied. Important risk factors are sexual intercourse without barrier contraceptive methods, a new partner or more than one partner, history of sexually transmitted diseases and being nulliparous or unmarried.

C. trachomatis can infect both women and men, and they have different symptoms and signs. In women, infection causes vaginal secretions, postcoital or intermenstrual spotting bleeding or spotting bleeding during administration of EPO, a hyperemic and friable cervix, urethritis, pelvic inflammatory disease, and, in sexually active women, pelvic pain and reactive arthritis. In men, it causes urethritis and urethral secretion, dysuria and, in sexually active men, epididymitis- orchitis and reactive arthritis.

Before starting an ART procedure or during a preconceptional counseling, it is important to consider the treatment of infection. Treatment should be performed only in specific cases, particularly in all patients with signs or symptoms associated with *C. trachomatis* infection (after diagnostic withdrawal, without laboratory test results) with particular attention to whether they are undergoing IVF cycles (risk of systemic dissemination during ISG, OLP, ISC, insemination) Treatment of an uncomplicated genital *C. trachomatis* infection can include 1 gr azithromycin in a single dose and 100 mg doxycycline twice a day for 7 days or erythromycin (2 gr/day for 7 days). For the treatment of genital infections involving high genitals, good tests of effectiveness are not available for women (salpingitis/pelvic inflammatory disease) or for men (epididymis-orchitis). SIGN guidelines for women recommend 100 mg doxycycline two times/day for a minimum of 10 days, or 400 mg ofloxacin two times/day associated with 200 mg metronidazole four times/day or 400 mg two times/day for 7 days, or 450 mg clindamycin four times/day. For men, the recommended treatments are 100 mg doxycycline two times/day for 7̃-14 days or 250 mg oxytetracycline four times/day for 7-14 days. The recommended treatment of mucopurulent cervicitis is 1 gr azithromycin in a single dose or 100 mg doxycycline two times/day for 7 days. It is very important to stop sexual intercourse until the end of the therapy (that means after 7 days of doxycycline or after 7 days from the azithromycin dose).

Chlamydia Infection during Pregnancy

If the genital infection by C. trachomatis occurs during pregnancy, it can produce several dangerous situations for the mother and the fetus. There can be intrauterine growth retardation, premature labor, or a low birth weight, premature rupture of the membranes, the death of the newborn, or postpartum endometritis. Treatment of uncomplicated C. trachomatis genital infection during pregnancy can be one of the following equally effective therapies: 500 mg erythromycin four times/day for 7 days (erythromycin estolate is contraindicated in pregnancy) or 500 mg amoxycillin 3 times/day for 7 days. Screening could be carried out in the first trimester of pregnancy as it could reduce complications such as premature delivery, while in the third trimester it could prevent transmission of the infection to the baby.

NEISSERIA GONORRHOEAE

Neisseria gonorrhoeae is a gram-negative diplococcus transmitted by direct contact with urethral, cervical, rectal, pharyngeal, and conjunctival secretions. In rare cases (<1%), infection spreads and can cause cutaneous lesions, arthritis, and tenosynovitis. Oral and pharyngeal infections can be asymptomatic, but infection can spread to the genitals by oral intercourse. In women, the genital infection is asymptomatic in 50% of cases; in the other 50% it produces leukorrhea, mucopurulent discharge, pelvic pain, and dysuria. In men, the genital infection is asymptomatic in 10% of cases, and in the other 90% it produces dysuria, mucopurulent discharge, and rarely (<1%) epididymitis and prostatitis. In woman neisseria infection, which is often associated with Chlamydia infection, is a frequent cause of infertility or subfertility.

VULVOVAGINITIS

Vulvovaginitis is characterized by leukorrhea, vulvar itch, and irritation. The most frequent causes of vaginitis are thricomoniasis, aspecific bacterial vaginosis,and candidosis. In rare cases, vaginitis is caused by *Chlamydia* or *Gonococcus*. Candida and Trichomonas don't usually involve high genital tract and they don't usually have role in infertility or miscarriage.

Nonspecific Bacterial Vaginosis

Nonspecific bacterial vaginosis is a syndrome caused by ,abnormal growth of anaerobic bacteria (*Prevotella*, *Mobiluncus*), *Gardnerella vaginalis*, or *Mycoplasma hominis* due to the detriment of the peroxide-producing *Lactobacilli*. Half the cases are asymptomatic and it is unknown if it is sexually transmitted. Bacterial vaginosis could be the cause of pelvic inflammatory disease and endometritis after invasive procedures (e.g., endometrial biopsy, hysterectomy, HSG, intrauterine device insertion, curettage). Between 9to23% of pregnant women are affected by bacteriaol vaginosis. Some data show an association between bacterial vaginosis and preterm delivery (RR 1.46.9), chorioamnionitis, PROM (RR 2.07.3), postpartum endometritis, and spontaneous miscarriage (RR 1.32.0). The management of bacterial vaginosis depend on several factors:

- all symptomatic women must be treated for bacterial vaginosis,
- asymptomatic women with a low risk of preterm labor must not be screened for bacterial vaginosis;
- asymptomatic women with a high risk of preterm labour should be screened for bacterial vaginosis during the first prenatal exam.

Herpes

Herpes is a recurrent infection caused by one of two herpes simplex viruses (HSV1 or HSV2). HSV1 causes up to 30% of the first-episode infections; HSV2 is more involved in recurrent cases. Clinical diagnosis is often ineffective because the typical lesions are not present (vesicles and ulcerations). Detection of virus in a culture is the most suitable diagnostic test in cases of active infection. Measurement of specific antiviral antibodies can distinguish between the two types of HSV and allows identification of asymptomatic infected patients. In the first clinical episode, the recommended treatment is oral doses of 400 mg acyclovir three times a day for 710 days or oral doses of 200 mg acyclovir five times a day for 7-10 days. In a recurrence, it is important to start the treatment within the first day of symptoms (with oral doses of 400 mg acyclovir three times a day for 5 days, oral doses of 200 mg acyclovir five times a day for 5 days, or oral doses of 800 mg acyclovir twice a day for 5 days). Long-term follow-up treatment can reduce relapses until a 3/4 oral dose of 400 mg acyclovir twice daily can be administered.

CONCLUSION

Infection play an important role in the reproductive outcome. As explained in this paper, some infection can be involved in the determining of infertility, they may complicate pregnancy causing miscarriage, ectopic pregnancy or in the late pregnancy PROM amd preterm birth. Moreover in other cases there are the concern of transmission infection both to the partner and the embryo. In case the couple request the ART a correct management of procedures is important to guarantee safety to child, partner, staff, and other patients in the IVF program. The ART procedures are usually organized into phases: (1) the diagnostic phase, intended to identify infections and other medical problems; (2) the procedures performed by physicians and biologists; and (3) the phase in

which executive and structural requirements are formulated for ART laboratories and fertility clinics. A correct diagnosis is useful to identify and inform the infected subjects. When an infection is detected, it is important to evaluate the infection risk of the partner and the embryo as well as the subsequent risks during pregnancy. Furthermore a strategy can be put in place before conception that will include obstetrical measures to reduce risks. Establishing guidelines for procedures also protects the staff (e.g., operators, doctors, biologists) and other patients. Precautions will also prevent or at least reduce risks of contamination.

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